

Applicants : DONG, Ke Wen et al.
U.S. Serial No. : 09/252,828
Filing Date : 19 February 1999
Page : 2

Prior to examination of the present Continuing Prosecution Application (CPA), Applicants respectfully request that the above-identified prior application be amended as follows:

In the Specification:

This is a Continuation of Application No. 09/252,828, filed February 19, 1999, which is in turn a non-provisional application of Application No. 60/075,079 filed on February 19, 1998.

In the Claims:

Please amend the claims as follows:

Please cancel Claims 25-38, 40 and 42-47 without prejudice.

Please add the following new claims:

-
48. (New) A purified recombinant glycopolypeptide of 65kd to 100kd that comprises approximately 40% to 60% carbohydrate by weight and that binds human spermatozoa at least 10 times as strong as an equivalent molar amount of mouse ZP3.
49. (New) The glycopolypeptide according to claim 48, wherein the glycopolypeptide is expressed by a human ovarian cell line.
50. (New) A purified recombinant glycopolypeptide having between 41 and 400 amino acids and having an active portion that binds human spermatozoa at least 10 times as strong as an equivalent molar amount of mouse ZP3, wherein the active portion comprises an

Applicants : DONG, Ke Wen et al.
U.S. Serial No. : 09/252,828
Filing Date : 19 February 1999
Page : 3

amino acid sequence that is more than 54% homologous with SEQ ID NO: 2 and has a predicted O-glycosylation site at a serine that corresponds to position 344 of the human ZP3 sequence.

51. (New) A glycopolypeptide according to claim 50, comprising a sequence from position 310 to position 345 of SEQ ID NO: 2 wherein at least one amino acid at a position selected from the group consisting of : (a)310; (b)320; (c)323; (d)326; (e)328; (f)329; (g)332; (h)334; (i)335; (j)337; (k)339; (l)341; (m)342 and (n)345 is substituted while preserving the human-species specific glycosylation pattern of the glycopolypeptide.
52. (New) The glycopolypeptide according to claim 50, wherein the amino acid sequence of the active portion is more than 75% identical with SEQ ID NO: 2.
53. (New) The glycopolypeptide according to claim 51, having between 41 and 300 amino acids.
54. (New) The glycopolypeptide according to claim 51, having between 41 and 200 amino acids.
55. (New) The glycopolypeptide according to claim 51, having between 41 and 100 amino acids.
56. (New) The glycopolypeptide according to claim 51, having between 41 and 65 amino acids.
57. (New) A purified recombinant glycopolypeptide of 65kd to 100kd that comprises 40% to 60% carbohydrate by weight and that binds human spermatozoa at least 10 times as strong as an equivalent molar amount of

I
Sub
K1

Applicants : DONG, Ke Wen et al.
U.S. Serial No. : 09/252,828
Filing Date : 19 February 1999
Page : 4

mouse ZP3, wherein the glycopolopeptide is obtainable by a process comprising the steps of:

- K1
- (a) transducing a cell from a human ovarian cell line with a polynucleotide that encodes a polypeptide comprising a sequence that is more than 54% homologous with SEQ ID NO:2;
 - (b) establishing a stable-transfected cell culture for producing the glycopolypeptide; and
 - (c) isolating the glycopolypeptide from the cell culture.

58. (New) The purified glycopolypeptide of claim 57 wherein the ovarian cell line of step (a) is selected from the group consisting of PA-1, EB2, CaoV-3, Cao V-4 OVCAR-3, SKOV-3, and SW 626.
59. (New) The purified glycopolypeptide of claim 57, wherein the polynucleotide of step (a) encodes a polypeptide comprising a sequence from position 310 to position 345 of SEQ ID NO:2 wherein at least one amino acid has been altered while preserving the human-species specific glycosylation of the glycopolypeptide.
60. (New) A purified glycopolypeptide that comprises carbohydrate and that binds human spermatozoa at least 10 times as strong as an equivalent molar amount of mouse ZP3, wherein the amino acid sequence of the glycopolypeptide comprises a sequence from position 310 to position 345 of SEQ ID NO: 2 wherein at least one amino acid has been altered while
- I1

preserving the human-species specific glycosylation of the glycopolypeptide.

- Sub 1
61. (New) A glycopolypeptide that binds human spermatozoa at least 10 times as strong as an equivalent molar amount of mouse ZP3 wherein the polypeptide portion of the glycopolypeptide is smaller than 25kd and includes a core region having a sequence shown in SEQ ID NO: 2 wherein at least one amino acid has been altered while preserving the human-species specific glycosylation of the glycopolypeptide.
62. (New) A glycopolypeptide having a polypeptide portion that is smaller than 10kd and which binds human spermatozoa with greater affinity than mouse spermatozoa, wherein the glycoprotein has a sequence comprising sequence position numbers 337 to 348 of SEQ ID NO: 2. Not capitalized.
- Sub 2
63. (New) (a) purified recombinant glycopolypeptide of 65kd to 100kd that comprises approximately 40% to 60% carbohydrate by weight and that can stimulate the acrosome reaction of human spermatozoa when co-present with the spermatozoa at a concentration of less than 1 µg/ml for a time period of less than one hour.
64. (New) The glycopolypeptide according to claim 63, wherein the glycopolypeptide is expressed by a human ovarian cell line.
- Sub 2
65. (New) A purified glycopolypeptide of 65kd to 100kd that binds human spermatozoa at a glycopolypeptide

concentration below 1 µg/ml and induce an acrosome reaction within one hour upon binding, wherein said glycopolypeptide comprises an amino acid sequence that is more than 54% homologous to the following sequence SEQ ID NO 2:

SerTrpPheProValGlnGlyProAlaAspIleCysGlnCysCysAs
nLysGlyAspCysGlyThrProSerHisSerArgArgGlnProHisV
alMetSerGlnTrpSerArgSerValSer.

66. (New) A glycopolypeptide comprising between 41 and 400 amino acid that binds human spermatozoa at a glycopolypeptide concentration below 1 µg/ml and induce an acrosome reaction within one hour upon binding, wherein said glycopolypeptide comprises an amino acid sequence that is at least 54% homologous to the following sequence SEQ ID NO 2:

SerTrpPheProValGlnGlyProAlaAspIleCysGlnCysCysAs
nLysGlyAspCysGlyThrProSerHisSerArgArgGlnProHisV
alMetSerGlnTrpSerArgSerValSer,

and wherein the fifth amino acid residue from the carboxyl terminus of said amino acid sequence of said glycopolypeptide is O-glycosylated.

67. (New) The glycopolypeptide of claim 66, wherein said glycopolypeptide comprises an amino acid sequence that is at least 75% homologous to the following sequence SEQ ID NO 2:

SerTrpPheProValGlnGlyProAlaAspIleCysGlnCysCysAs
nLysGlyAspCysGlyThrProSerHisSerArgArgGlnProHisV
alMetSerGlnTrpSerArgSerValSer.